

Research Article

Designing biosurveillance using target analysis for a cryptic invasive species, the brown treesnake (*Boiga irregularis*)

Melia G. Nafus¹, Marijoy C. Viernes¹, W. Trey Dunn², Jennifer A. Fike³, Sara J. Oyler-McCance³, Joseph Cruz⁴

¹ U. S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, Hawai'i, USA

² U.S. Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office, Saipan, Commonwealth of the Northern Mariana Islands, USA

³ U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado, USA

⁴ Division of Fish and Wildlife, Saipan, Commonwealth of the Northern Mariana Islands, USA

Corresponding author: Melia G. Nafus (mnafus@usgs.gov)

Abstract

Biosurveillance is especially important for invasive species that are difficult to control or remove once established. Target analysis, or the strategic evaluation of factors that affect or improve detection of organisms, is one approach to improve biosurveillance over time. Due to their ecologically and economically devastating effects, brown treesnakes (*Boiga irregularis*), an invasive snake in the United States territory of Guam, have a dedicated biosecurity program to prevent their spread. Guam has been occupied by brown treesnakes since the 1940s, and they are distributed throughout the island. The neighboring Saipan Island, otherwise known as Saipan, participates in biosecurity programs designed to prevent their establishment. We evaluated biosurveillance protocols within rapid response and detection exercises for brown treesnakes. Our goal was to identify, account for, and reduce potential error when inferring absence from lack of detection using data collected in Guam and Saipan. We evaluated 1) whether brown treesnake detection probability was density dependent; 2) whether using terrestrial environmental DNA (eDNA) sampling detected snakes in occupied areas; 3) the likelihood brown treesnakes would have been detected in Saipan, given sampling effort during a biosurveillance exercise, and whether lizards could be used as a proxy on snake-free islands for estimating factors that affect detection; 4) the effect of transect design on pacing and subsequently pacing on lizard counts; and 5) the importance of observer training in surveying for cryptic reptiles and completing broad versus targeted bioinventories. We found that brown treesnake detection probability was density dependent when using visual surveys and declined with abundance in Guam, supporting that greater effort was required to have high confidence that lack of detection supported absence in areas with low snake densities. The terrestrial eDNA sampling protocol we developed was effective at detecting snakes in occupied locations in Guam and may be a complementary tool. However, further validation of eDNA protocols would still be required for early detection application. We also found that among trained observers, the ability to detect lizards was positively predictive of the number of snakes they detected at the standard snake densities in Guam. Thus, lizard detection appeared to be a reasonable proxy for evaluating visual search protocols for snakes on snake-free islands. Overall, creating easy pace-setting relationships between time and distance (i.e., 10 m per min), with flagging that clearly marked location on a transect, greatly reduced variability in search pace across survey nights. Observers that were continuously trained to maintain brown treesnake search profiles detected twice as many, on average, cryptic reptiles compared to individuals who had never received training, which was not true for the non-reptiles (birds and small mammals). Additionally, conducting broad bioinventories with many target species reduced detection of the target species, and this was increasingly true as years since the observer completed training for the target species increased. We



Academic editor: Samuel Ward

Received: 9 April 2025

Accepted: 21 September 2025

Published: 6 January 2026

Citation: Nafus MG, Viernes MC, Dunn WT, Fike JA, Oyler-McCance SJ, Cruz J (2026) Designing biosurveillance using target analysis for a cryptic invasive species, the brown treesnake (*Boiga irregularis*). NeoBiota 105: 1–23. <https://doi.org/10.3897/neobiota.105.155408>

Copyright: This is an open access article distributed under the terms of the CC0 Public Domain Dedication.

thus found that designing biosurveillance exercises to evaluate search protocols can help improve or refine survey methods to reduce noise in the data, and accounting for that can improve the estimated confidence that failure to detect an invasive species infers absence.

Key words: *Boiga irregularis*, density dependence, early detection and rapid response, eDNA, observer training, protocols, target species

Introduction

Invasive species threaten many organisms and ecosystems, and the prevention of their spread is critical in an era of major biodiversity loss (Doherty et al. 2016; Mollot et al. 2017). Invasive species mitigation measures include strategies that prevent, detect, and control existing populations (Marbua et al. 2014). The efficacy, practicality, and economy of invasive species management are determined by the tools available for removal, funding, political support, social risk or license for action, and timeliness of a response to a novel population (Epanchin-Niell et al. 2010; Marbua et al. 2014; Alvarez and Solis 2018; Roberts et al. 2018). After prevention, early detection, which includes biosurveillance to detect incipient populations and rapid removal to prevent spread, can be the next most economical approach (Alvarez and Solis 2018). Once introduced, the stage at which an invasion is detected can be a large driver of the costs associated with species management.

Biosurveillance or early detection programs operate efficiently by developing protocols that consider ecological, entry pathway, methodological, or other factors that affect the probability of detection under the constraint of finite resources. For example, strategic spatial focus to reduce the extent of coverage can be one approach, with biosurveillance prioritized near high-risk areas such as ports of entry and distribution warehouses (Lodge et al. 2006). Targeted biosurveillance at sites with a high probability of introductions can be a more economical approach to mitigating costs associated with invasive species management (Hauser and McCarthy 2009). For species with high costs to control and long lag periods between establishment and spread, greater investment in early detection surveys is suggested to be more sustainable and efficient than other mitigation measures (Mehta et al. 2007; Epanchin-Niell et al. 2012). There are various methods that can be used when conducting biosurveillance, including the use of environmental DNA (eDNA), chemical approaches (detection dogs, chemical lures), remote sensing, and citizen science, along with more traditional options (Larson et al. 2020). Tools such as eDNA, camera traps, and citizen science can be essential for enhancing detection of cryptic invasive species, in particular (Jarić et al. 2019). Target analysis, or strategic protocols for detection based on space or time, can help refine the mode and approach to selecting species-specific protocols built upon the biology, ecology, or species behavior (Morissette et al. 2020). Species-specific information can be combined with knowledge of the technologies available or their practical constraints to guide when, where, or how biosurveillance protocols are implemented and thus maximize probability of detection (Morissette et al. 2020). For rare or cryptic species, comparative analysis of individual technologies can identify seasonal or other factors that may be necessary to consider when designing a biosurveillance program (Vine et al. 2009). Spatial configuration, structure, and duration of surveillance are thus each important considerations when developing sustainable and economical early detection programs.

Invasive snakes are recognized as one of the more ecologically harmful invasive vertebrates, and ecological cascades often follow their introductions (Savidge 1987; Dorcas et al. 2012; Kraus 2015; Silva-Rocha et al. 2015; Willson 2017; Piquet and López-Darias 2021). Invasive snakes are cryptic and often have low detection probabilities (Christy et al. 2010; Nafus et al. 2020). Snakes can also have decades-long lag times between establishment and notable ecological effects (Savidge 1987; Willson 2017). There are few effective control tools for snakes, which are also costly to manage, and there are no established eradication techniques at meaningful scales (Vice and Pitzler 2000; Pitt et al. 2005; Nafus et al. 2024). Thus, invasive snakes, although difficult to detect, are theoretically strong candidates for early detection and rapid response programs.

The brown treesnake (*Boiga irregularis*) has a formal biosecurity program in its invasive range (Vice and Pitzler 2000; Stanford and Rodda 2007). Brown treesnakes were introduced to Guam (Guåhan in CHamoru) shortly after World War II and caused major ecological change, as well as harmful human effects following their introduction (Savidge 1987; Fritts 2002; Rodda and Savidge 2007; Caves et al. 2013). Prevention of their spread in the Pacific region is managed through a multi-agency interdiction program that includes the use of traps, visual searches, and detector dogs to prevent snakes from moving in cargo (Vice and Pitzler 2000). The brown treesnake Rapid Response Team (RRT) is a multiagency program that relies on a core group of continuously trained experts and intermittently trained personnel from cooperating state and federal agencies, as well as universities and other nonprofit institutions (Stanford and Rodda 2007). Training of cooperators is set at 25 h of visual surveys, occurring approximately every 2 years, and since the implementation of various biosecurity programs, the number of snakes detected off the island of Guam has decreased (Stanford and Rodda 2007). Once a rapid response is deemed warranted, surveys rely on both trained and untrained personnel to complete visual surveys and/or trapping adjacent to the sighting report.

As part of a biosurveillance effort that includes early detection initiatives, the United States Geological Survey (USGS) partnered with the Commonwealth of the Northern Mariana Islands (CNMI) Division of Natural Resources to trial a “mock” response. The purpose of this effort was to build interagency response readiness, engage in surveillance, and evaluate response protocols to identify efficient, standardized approaches and biases within established search or detection protocols. To test assumptions built from ecological information and detection methods that may improve survey protocols (Table 1), we used data collected on Guam and Saipan Island (hereafter Saipan). Recently, approaches have been developed to estimate confidence that lack of detection during biosurveillance supports absence of an incipient brown treesnake population; however, methods relied on detection estimates formed from the population densities typical in Guam, with an assumed correction factor for the lower densities expected for populations early in the invasion curve (Yackel Adams et al. 2021). For many species, detection probability is density dependent, or a factor of abundance (Lebreton and Gimenez 2013), but the exact relationship is likely species-specific. Likewise, recent work has suggested that the use of eDNA can meaningfully improve detection for low-density or cryptic reptiles, although its use does not always improve detection (Hunter et al. 2015; Rose et al. 2019; Kyle et al. 2022). In general, statistical assumptions are improved by reducing noise in the data created by protocol variance (Isaac et al. 2014). In the case of visual surveys, factors such as observer, pace, location, and

accuracy of search image can all affect success in wildlife detection (Henke 1998; Isaac et al. 2014; Lardner et al. 2019a; Lardner et al. 2019b). Thus, in addition to exploring basic detection and ecology questions that may inform interpretation or tool application, we were also interested in understanding how transect design, ability to focus on a target species, and training level affected variance in the data produced. Because we wanted to use data gathered during biosurveillance activities in islands without known snake populations, we also investigated whether other organisms that were present might function as a proxy for snakes. Specifically, as lizards in Guam are also cryptic and have behavioral patterns that are more similar to snakes than other vertebrates or invertebrates, we were interested if a trained observer’s ability to detect lizards correlated with their success in detecting snakes.

Based on the above factors, which can materially affect interpreting lack of detection as absence, as well as the interpretive use of data in areas without snakes, we focused on five questions. In order of their presentation in the text, we asked the following: 1) whether detection probability for brown treesnake populations was density dependent; 2) if eDNA protocols to detect arboreal brown treesnakes could be developed; 3) what was the probability that at least one snake would have been detected during the biosurveillance exercise in Saipan, and whether searcher ability to detect snakes predicted their lizard counts; 4) if simplifying transects and pacing structure into simple time-to-distance calculations improved pacing standardization; and 5) how training and experience affected detecting cryptic reptiles, especially while conducting multispecies bioinventories.

Table 1. The application of structured target analysis to strategically evaluate known or presumed sources of error related to methods of detecting brown treesnakes (*Boiga irregularis*) can help refine search protocols if hypotheses are tested during a biosurveillance exercise in Saipan and Guam.

Methods	Sources of error	Hypotheses tested
Visual surveys	<i>Observer</i>	
	Training level	Ability to detect cryptic reptiles will increase with training.
	Differences in skill	
	<i>Equipment</i>	
	Luminosity	
	<i>Methodology</i>	
	Pace	
	Transect or grid design	Edge surveys when snake population densities are lower will have reduced detection probabilities.
	Timing or duration	
	Data collected	Broad biological inventories with multispecies targets will reduce detection of target species.
	Level of effort	
	Location sampled	
eDNA	<i>Laboratory analysis</i>	
	Insufficient assay sensitivity	
	Lack of target specificity DNA degradation	
	<i>Sample collection</i>	
	Contamination	Water-holding plants with preferential use by brown treesnakes will improve detection probability over edge-based visual surveys alone.
	Level of effort	
	Location sampled	

Materials and methods

Study sites

The Mariana Islands have a wet, tropical marine environment with mean temperatures of 30–32 °C and peak rainfall occurring from July–October (Yeo et al. 2023). Habitat is a mix of coastal strand, limestone or degraded limestone forest, and savannah (Fosberg 1960). Guam and Saipan are separated by approximately 185 km of ocean (Fig. 1).

In Guam, one study site included a 5-ha snake enclosure located in northern Guam (NWFN: 13°37'N, 144°51'E) used to collect visual survey data (Fig. 1). NWFN was enclosed by a barrier that prevented ingress and egress of brown treesnakes (or snakes), which allowed complete censusing of the population (Rodda et al. 2007). The primary forest was degraded limestone forest, dominated by *Leucaena leucocephala* and *Hibiscus tiliaceus* on a limestone karst substrate (Nafus et al. 2018). Within NWFN, we maintained 29 transects separated by 8 m to complete visual surveys. Observers completed a single 220-m forest edge transect at a 10 min pace (22 m/min) and searched in a single direction. We completed nocturnal visual surveys with observers equipped with high-powered headlamps (Lupine, Wilma, Lumens = 3100) from 24 October 2016–27 April 2023. All observers were trained with 25 h of visual survey training for brown treesnake detection prior to working in NWFN. We completed surveys of all transects in the enclosure each night. When a snake was detected, we attempted to capture the snake. We uniquely marked and released captured snakes to create a known population. Based on intensive mark–recapture approaches, we had continuous knowledge of the true abundance of snakes in the enclosure throughout the period of study. The methods

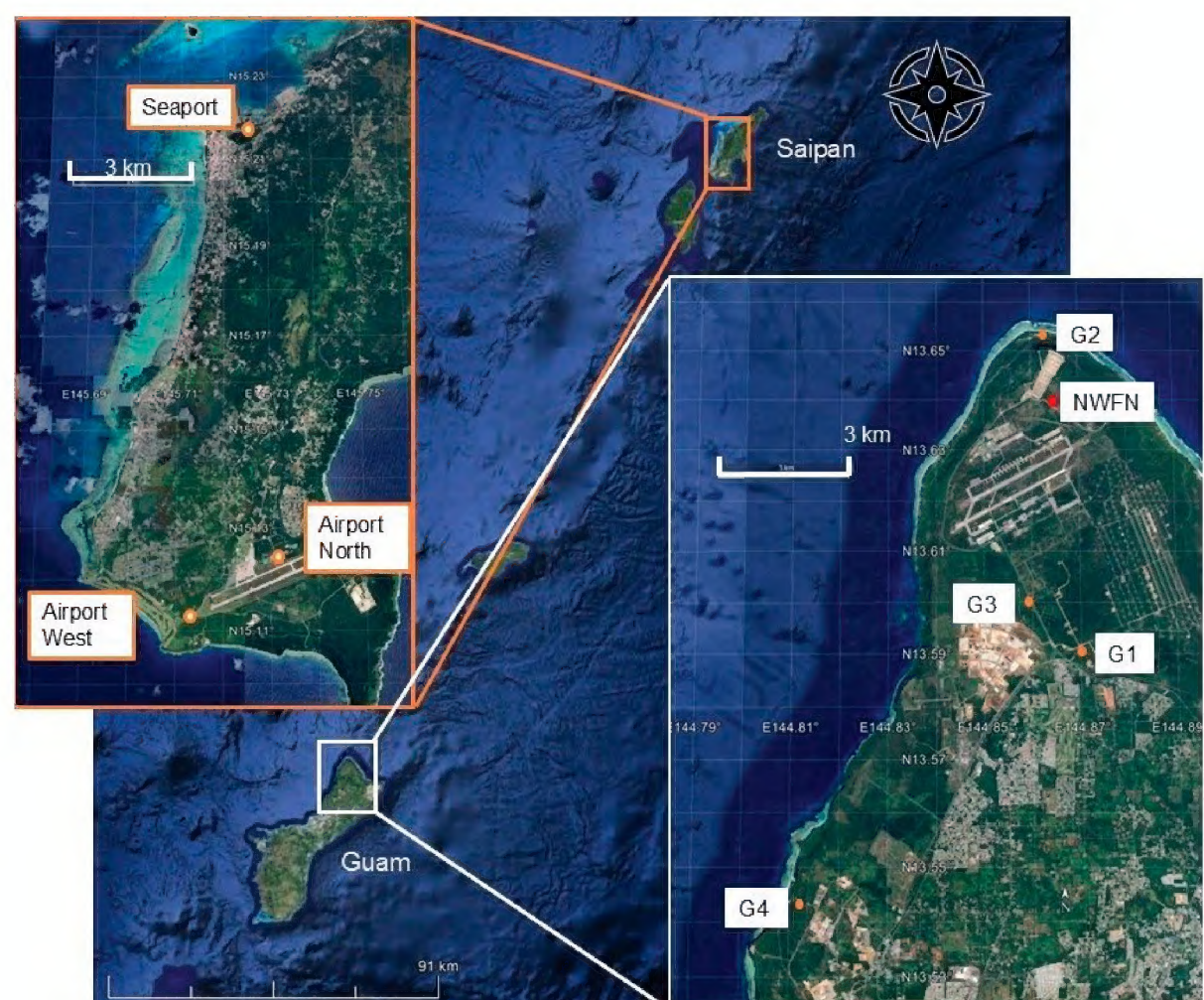


Figure 1. Images of Guam, outlined in white, and Saipan, outlined in orange, located in the Mariana Islands. Visual transects in Saipan (Airport West, Airport North, and Seaport) are labeled with yellow dots. The visual survey transects in Guam are labeled with a red dot (NWFN), while eDNA sites are labeled with orange dots (G1–G4). Image created on Google Earth using SIO, NOAA, U.S. Navy, NGA, and GEBCO data.

of estimating presence were described in detail in the affiliated data release (Nafus et al. 2023). From 2017–2022, we removed snakes through a variety of approaches that reduced the density of the population from 23.4 to three snakes/ha (removal methods were described in detail in Nafus et al. 2024). All snake handling was completed in accordance with Institutional Animal Care and Use (IACUC) protocol 2017-03 granted through the USGS Fort Collins Science Center.

In northern Guam, we also selected four roadside locations (13°32'N, 144°51'E; 13°35'N, 144°51'E; 13°32'N, 144°48'E; 13°35'N, 144°48'E) to collect eDNA samples (Fig. 1). At each eDNA sampling location, we selected four separate trees, which we refer to as sites hereafter. We had no known density of snakes at eDNA sites, but all of Guam is presumed to be occupied by snakes. The four general geographical locations selected in Guam for eDNA sampling were degraded limestone forests on karst substrate along roadsides or previously cut paths, but outside the NWFN enclosure.

We completed visual surveys in Saipan at two ports of entry, a marina for private vessels (Seaport: 15°12'N, 145°46'E) and two sites adjacent to the airport (North: 15°6'N, 145°43'E; West: 15°7'N, 145°43'E) (Fig. 1). All transects occurred along open forested areas within these sites. We selected biosurveillance sites based on their proximity to vessels that arrive directly from Guam and credible brown treesnake sightings that had occurred in adjacent areas in the last decade (Yackel Adams et al. 2021). Seaport habitat was primarily disturbed coastal strand dominated by *H. tiliaceus*, ironwood (*Casuarina equisetifolia*), coconut palm (*Cocos nucifera*), and other miscellaneous tree species. The airport habitat was primarily disturbed limestone forest, which was dominated by *L. leucocephala*, *Albizia* spp., and flame trees (*Delonix regia*). We established all transects along current or historical (overgrown but traversable) roadsides at the edge of the forest.

Density-dependent detection probability, Guam

We used an occupancy analysis to estimate detection probability, from which we calculated the number of site visits necessary to detect a snake with a 99% confidence level when conducting edge-only transects at high- and low-density snake populations in NWFN. We used 22 survey nights from 24 October–30 November 2016, when snake density in NWFN was 23.4 snakes/ha (high), and 22 survey nights from 17 August–19 October 2022, when snake density was at 3–5 snakes/ha (low), to estimate detection probability at the specified abundance (d) from nocturnal visual surveys (Table 2). We only used snake detections from a single edge

Table 2. The density of a known population of brown treesnakes in a 5-ha snake enclosure in northern Guam by year and month, with the number of site visits that occurred each month and whether density was considered low or high.

Year	Month	Site visits	Snake density	Density category
2016	October	5	23.4	High
	November	17	23.4	High
	December	6	23.4	High
2022	August	10	5.2	Low
	September	11	4.0	Low
	October	4	3.2	Low
	November	3	3.0	Low

transect survey in NWFN, with all data from other transects discarded for each site visit. We selected an edge transect because biosurveillance for brown treesnakes on uninvaded islands typically occurs on forest edges due to high costs and time requirements for cutting transects through jungle. There were only two edge transects, so we selected the transect that was degraded limestone forest, rather than primarily *L. leucocephala* stands. We were unable to estimate detection probability at densities lower than three snakes/ha due to failure in model convergence, which necessitated filtering detection data generated after 2022.

To estimate the number of forest edge site visits necessary for a 99% confidence interval that at least one snake would be detected, if snakes were present, we used a modified version of the formula (McArdle 1990), as solved for k :

$$k = \ln(1 - \alpha) / \ln(1 - d)$$

where:

α = the probability that an organism is detected at a given abundance based on effort,

d = the probability of detecting the organism at a specified abundance,

k = surveillance effort as measured by a single transect or sampling point.

We estimated d using the “unmarked” package in R (MacKenzie et al. 2003) from the visual transect surveys in NWFN and set $\alpha = 0.99$. Data used in this analysis were extracted from Nafus et al. (2023).

eDNA for terrestrial detection of brown treesnakes, Guam

The terrestrial eDNA method presented here is meant to be a proof-of-concept and was not tested or validated (e.g., reporting limits of detection, quantifying field contamination) at standards desired for implementation as a management tool. We first tested our eDNA assay and collection method for brown treesnakes using four sampling approaches that included: a negative control, which was a bin of tap water filled from the faucet; a positive control of tap water containing 1) snake feces; 2) a snake shed; 3) a live snake in a bin with a water bowl for the preceding 24 h; and 4) an outdoor ornamental plant that had water with snake feces poured over it. The bins of water were kept sealed and stored at ambient outdoor temperatures, and the plant was exposed to the elements. We collected samples at 0, 3, 6, 12, 17, and 21 days post exposure using standard paper towels that we dipped into the water or wiped across the leaf surface. We air-dried paper towels in a climate-controlled building without snakes and stored them at room temperature until processed.

We then collected eDNA from field samples using paper towels to swab *Pandanus tectorius* leaves. Brown treesnakes have a documented preference for using *Pandanus* as refugia (Tobin et al. 1999, Boback et al. 2020). *Pandanus* crowns also hold water in small pools that allow for absorption. Thus, we hypothesized that swabbing water pools or wet leaves of *Pandanus* was a potential method to easily collect brown treesnake DNA in the Mariana Islands. To collect DNA, we ran the paper towel down individual leaves by hand until the entire towel was moist. We changed gloves between samples, and each paper towel was stored in an individually sealed plastic sandwich bag. At the close of the field day, the bag was opened to allow the towels to dry and then sealed once dry. At each roadside sampling location, we selected four different *Pandanus* trees that bordered pre-existing

trails or roads, which we refer to as sites for occupancy analysis ($N = 16$). A single person returned to each plant and collected samples daily for 3 days ($N = 48$) in February 2020 (representing three replicates). We swabbed the same leaf clusters (a *Pandanus* crown) each day, but a given swab covered more than one leaf as needed to ensure the entire towel was saturated with water. Specifically, if swabbing multiple leaves in a single crown was required to saturate the towel on the first day, we swabbed all the same leaves on future days.

We removed a subsection (approximately 5×5 cm) of each paper towel sample with sterile scissors and forceps and processed as described below. The remaining paper towel was returned to its original sample bag and archived at -20 °C. DNA from each paper towel subsection was extracted using the Qiagen QIAshredder/DNeasy Blood and Tissue Kit method as described in Goldberg et al. (2011). This method followed the manufacturer's protocol with the following modifications: (1) samples were incubated at 65 °C for 10 min after adding Buffer AL; (2) samples were eluted in 100 µL warm Buffer AE followed by 5 min incubation at room temperature. An extraction negative was processed along with each batch of samples (23 samples and one extraction negative) to monitor for contamination.

We ran samples on a brown treesnake-specific qPCR TaqMan assay developed in our lab (assay name: BTS2, forward primer: ATCACAATCACATGAGCTGCAACT, reverse primer: GAAGTATAGGAGGGAGGTGATTTGTC, probe: AGTAGAACCACCATTAC). The qPCR was performed using 2 µL of template DNA in a 20 µL reaction containing 250 nM of TaqMan probe (labeled with 6-FAM and MGB [5' and 3', respectively]) in $1\times$ PCR TaqMan Fast Advanced Master Mix (Applied Biosystems), 500 nM of each primer, 0.4 µL of Exo IPC DNA, and 2.0 µL of Exo IPC Mix. The reactions were run on an Applied Biosystems StepOne-Plus Real-Time Polymerase Chain Reaction (PCR) system with the Fast quantitative PCR (qPCR) profile consisting of 50 °C (2 min) and 95 °C (2 sec), followed by 40 cycles of 95 °C (1 sec) and 60 °C (20 sec). We ran all samples, including the extraction negatives and qPCR negatives, in triplicate with a seven-point standard curve developed from 1:10 serial dilutions of brown treesnake liver tissue DNA quantified on a Qubit 2.0 Fluorometer (Life Technologies). A TaqMan Exogenous Internal Positive Control (IPC; Applied Biosystems) was used to monitor for PCR inhibition. If there was a shift in the cycle threshold (CT) of the IPC, or if the IPC failed to amplify in any of the three replicates, then we considered that sample inhibited. We cleaned samples identified as inhibited with a Qiagen DNeasy PowerClean Pro Cleanup Kit and then re-ran them on the qPCR assay for three replicates.

If there was no amplification in any of the three replicates, we classified the sample as negative. If there was amplification in all three replicates, the sample was classified as positive. During the initial validation phase, we focused on whether a sample was positive in the majority of replicates—i.e., if two out of three replicates amplified, the sample was considered positive. In the second phase of the project, when sampling *Pandanus* trees, we followed a more detailed protocol for ambiguous results. If a sample amplified in only one or two out of the three replicates, we followed the approach outlined in Goldberg et al. (2013). Briefly, we re-ran those samples with three additional replicates: if any of the three re-run replicates amplified, the sample was classified as positive. If the sample had amplified only once in the first round and none of the re-run replicates amplified, it was classified as negative. If the sample had amplified in two out of three replicates in the first

round but failed to amplify in the re-run, we conducted a third round of three replicates to confirm the negative result.

Although the eDNA methods were primarily pursued to investigate whether we could develop swabbing protocols that detected brown treesnake DNA, we also used site visits to calculate d from eDNA in areas known to be occupied by the species. We did not validate protocols for using eDNA for application in areas without confirmed populations of brown treesnakes. This is interpreted as a fully separate measure of estimated detection probability from visual surveys, with each plant treated as a separate site ($N = 16$) and each sampling day treated as a site visit ($k = 3$ per site or 48 overall). Given an assumption of 100% brown treesnake occupancy in Guam, we evaluated d for eDNA in Guam to estimate error rates and evaluate if it was substantially lower than for visual surveys prior to population reductions in NWFN.

Probability of snake detection in Saipan and using lizards as a proxy for snakes in Saipan and Guam

As indicated, lizards are not the same as snakes, although they are typically more like snakes than other vertebrates. Thus, we evaluated whether observer acuity in detecting snakes predicted their success in counting lizards. For each observer, we calculated the average sightings of lizards or snakes per km searched by an observer across all nights they completed surveys in NWFN from 24 October 2016–31 March 2020 (Nafus et al. 2023). Due to substantive declines in the snake population and staffing turnover, we omitted data collected after March 2020. Each observer ($N = 27$) thus had a single count per unit effort (CPUE) for lizards and a separate CPUE for snakes. We used a negative binomial generalized linear regression to predict whether observers' lizard detections per km predicted their snake detections. We selected a negative binomial fit based on evidence of limited overdispersion of the conditional distribution in the data (Lindén and Mäntyniemi 2011). We transformed snake data to whole numbers by multiplying the dataset by 10. After validating a relationship between these variables, we proceeded with using lizards as a proxy for snakes.

To generate data used to evaluate the probability of having detected a snake in Saipan during a mock response, we completed visual surveys from 16–30 October 2023. In total, 29 observers completed visual surveys at three sites: Seaport ($N = 12$), Airport West ($N = 9$), and Airport North ($N = 19$) (Fig. 1). Each established transect was set at 300 m, with a single pace of 30 min. We placed flagging every 10 m (10 m per min search pace) with a single band of reflective tape. Every 50 m we used double-reflective flagging strips. A different color of flagging was used to mark the start and end of the transect. We instructed observers that 1 min was required to go from one 10-m flag to the next and each 50-m interval should reflect approximately 5 min of search time, with the entire transect completed in 30 min. An equal number of observers was assigned to each site every survey night. Observers completed 4–6 transects per survey night. Data used in this analysis were published in Nafus and Viernes (2025).

During 2023, we broadly controlled for observer training by setting three general categories: continuous ($N = 12$), intermittent ($N = 12$), or untrained ($N = 4$). Continuously trained observers received 25 h of visual training and continuously gained experience by conducting nocturnal surveys for snakes in Guam, usually

more than one time per week, or were otherwise regularly engaged in reptile surveys. Intermittent observers had received 25 h of nocturnal visual training for brown treesnakes, with 0.1–5.1 years between their training and October 2023 ($\mu = 2.6$ years). Untrained observers were individuals that had not received training for brown treesnake detection in Guam. Both intermittent and untrained observers also did not regularly participate in alternative reptile surveys. On each night, a continuously trained observer was paired with an intermittently or untrained observer, and transects were replicated by observer pairs in a stratified-random order. Observers used two headlamp types: Wilma (continuously and some intermittently trained observers) or HP30 (Fenix, lumens = 900, some intermittently and untrained observers).

We used a paired study design to complete transects. Observers alternated between counting any lizards, small mammals (shrews, mice, or rats), and birds observed (ALL, $N = 335$) or only counting a single species of lizard by transect (LS, $N = 327$). The single species selected was a non-native skink, *Lamprolepis smaragdina* (emerald tree skink), which of the species available was closest in shape to a snake. Emerald tree skinks are, however, bright green instead of brown. Each night, we randomly assigned the first transect type (ALL or LS) to the continuously trained observer, and the opposite transect type was assigned to their partner. Thereafter, order alternated between ALL and LS to control for time-dependent effects on detection for each observer. We completed all work in accordance with CNMI Division of Fish & Wildlife permit #24S-012N and USGS Fort Collins Science Center IACUC protocol #2021-01. We discarded data from 10 transects based on lack of compliance with study protocols (the full transect was not completed, an observer drove instead of walked, or the observer transitioned between ALL or LS counts partway through the transect).

Using the 2023 dataset, we used the McArdle model ($\alpha = 1 - [1 - d]^k$) to calculate the probability that we would have found at least one snake based on the number of site visits (k) for each site and the low-density detection probability (d) calculated from NWFN visual surveys when answering question 1.

Transect structure and pacing, Saipan

In addition to the data created in 2023, we used a previously published, historical dataset generated during a brown treesnake Rapid Response Team deployment in 2016 to evaluate how transect structure affected pacing and lizard detections. Specific methods for surveys are previously described (Yackel Adams et al. 2018; Lardner et al. 2019a), but briefly, pacing was set to approximately 1 h per transect or roughly 7.5 m per min (Yackel Adams and Lardner 2020). Transect length was also variable, ranging from 400–600 m for transects located at the airport. There were six transects located at the airport during the 2016 surveillance. Each transect was surveyed between 6 and 17 times by observers, and observers consisted of a mix of experience levels. Transects were flagged with regular spacing and had no visual differentiation in flagging by distance or location. Observers counted all lizards, small mammals, and birds seen during surveys. Exact transect placement was variable between 2016 and 2023. Additionally, in both years paired observers could sometimes see their partner while surveying a transect and other times could not. Because transects were on edge, most transects were linear in sequence, and individuals had poor visibility into the pace of their partner.

We completed two analyses to evaluate whether increasing the pace to make pacing easier altered detection success. To minimize potential habitat or survey effects on detection, we omitted Seaport and LS data from the 2023 dataset. Thus, this analysis focused only on airport surveys for all lizards, small mammals, and birds during 2016 and 2023. For the first analysis, we completed a Wilcoxon rank sum test on the variability in pacing (m/min by year) during 2016 and 2023 to evaluate whether pacing was more even and standardized in 2023 relative to 2016. We used pace (m/min) and year as the test variables. As a separate analysis, we tested whether the increased pace set in 2023 to make time-to-distance ratios easier to calculate affected detection of cryptic reptiles. For this analysis, we used a generalized linear mixed effect model with a Poisson distribution using the lme4 package in R (Bates et al. 2013). We set the total lizards counted for all species within a transect as the response. We included transect identity (unique name) and observer identity as random effects. We included pace (meters surveyed per min) and observer training level as fixed effects in the model. For simplicity, we converted observer training into a binary factor where a value of “1” was a continuously trained observer and “0” was an individual that had no or intermittent training. We included an interactive term between pace and observer training to evaluate whether there was an experience- or pace-based effect on lizard detections.

Observer experience effects on cryptic reptile detection and multispecies bioinventories

Using data from all the 2023 Saipan transects, we completed two analyses to evaluate the importance of training and skill on wildlife or cryptic reptile counts (Nafus and Viernes 2025). The two analyses evaluated whether observer training and duration of participation affected detection of (1) cryptic reptiles or (2) other small vertebrates (small mammals and birds). We combined birds and small mammals as a single response based on their comparatively lower encounter rates. We included counts of reptiles, birds, and mammals as a response in the generalized linear mixed effect models, using a Poisson distribution in the lme4 package in R (Bates et al. 2013). We used observer training as a linear fixed effect, which was assigned as a continuous variable based on years since participation in active surveys or receiving training for snakes. Values ranged from 0 (continuously trained) to 10 (no training). For intermittent observers, we subtracted the last date in which they participated in a brown treesnake Rapid Response Team training course from October 2023. We also included the number of days (search days) the observer participated in the mock response as a cumulative continuous fixed effect, such that the number for their first night of surveys was one and the number for their second night was two, and so on until they stopped participating. We again included transect identity, observer identity, and site as random effects to control for variability across each factor.

As the last analysis, we explored whether detection of the target species was affected by conducting broad vertebrate bioinventories as a function of observer training level. We included all 2023 data in a generalized linear mixed effect model with a Poisson distribution using the lme4 package in R (Bates et al. 2013). We included survey type (ALL or LS) as a categorical fixed effect and the number of emerald tree skinks detected per survey as the response. We also included training level as a continuous fixed effect, with an interactive effect between survey type

and training. We included search days as a continuous fixed effect. We included transect identity, observer identity, and site as random effects to control for their known variance. We used $\alpha \geq 0.05$ to select significance and completed all analysis in R version 3.6.1 (Vienna, Austria).

Results

Density-dependent detection probability, Guam

In total, we completed 30,762 transects across 557 survey nights in NWFN, which we filtered to 44 transects over 44 nights. Occupancy analysis supported density-dependent detection probabilities for brown treesnakes (Table 3). An ~80% reduction in density reduced d by roughly 67% along forest edge transects (Table 3). This equated to a 220% increase in predicted effort as measured by the number of site visits estimated to achieve a 99% probability that at least one snake would be detected at the reduced density (Table 3).

Use of eDNA for terrestrial detection of brown treesnakes, Guam

During initial testing, all eDNA negatives were negative and most positives tested positive for up to 21 days of sampling (Table 4). Every sample except those collected on the plant exposed to water with brown treesnake fecal and shed matter on day 12 tested positive for snake eDNA, yielding a 8% false negative rate after DNA exposure (Table 4).

Table 3. The detection probability (d) and 95% confidence interval (CI) calculated from an occupancy analysis of brown treesnakes (*Boiga irregularis*) in a 5-ha enclosure in Northern Guam, with a known density of individuals and the number of site visits (k) to have a 99% probability of detecting at least one snake based on d .

Snake density (No./ha)	$d \pm 95\% \text{ CI}$	k (99%)
23.4	0.21 ± 0.08	20
3–5.2	0.07 ± 0.05	64

Table 4. Validation of brown treesnake eDNA assay primers for eDNA. If at least two of three laboratory replicates tested positive (+), we classified the sample as positive; otherwise the sample was classified as negative (-). Each material was sampled six times over 21 days. The negative was tap water that was not exposed to brown treesnakes. Positive samples were tap water exposed to either snake feces, a shed, or a live snake for 24 h and stored at room temperature for 21 days, as well as an outdoor plant that had tap water with snake feces deposited on it.

	Days post set up					
	0	3	6	12	17	21
Negative	-	-	-	-	-	-
Positives						
Water - Feces	+	+	+	+	+	+
Water - Shed	+	+	+	+	+	+
Water - Snake	+	+	+	-	+	+
Plant - Feces	+	+	+	-	+	+

All four locations in Guam were confirmed to be occupied by brown treesnakes based on at least one site (*Pandanus* tree) testing positive for brown treesnake DNA. However, there was more variability in returning a positive sample across the four sites, as well as each of the three replicates run for field samples (Table 5). Based on an assumption of 100% occupancy by snakes and thus potential exposure to DNA at all sites, the estimated false negative error rate was ~65% in a single sampling event in a field setting. In other words, 65% of the total samples run were negative for brown treesnake eDNA, even though the area was occupied by snakes ($d = 0.35 \pm 0.07$). Using the McArdle (1990) equation, we estimated that 11 site visits were required for a 99% probability of detecting snake DNA in *Pandanus* on the forest edge at the unknown density of snakes in Guam’s forests.

Probability of snake detection in Saipan surveys and using lizards as a proxy for snakes in Saipan and Guam

We found a strong positive relationship between observer counts of lizards and their average rate of detecting a snake as measured by sightings per km (Fig. 2). For each additional average lizard sighted per km, an observer had a 1.1 times improvement in their average rate of detecting snakes ($\beta = 0.06$, 95% confidence interval [CI] = 0.02, 0.10, $z = 2.27$, $P = 0.02$).

Table 5. The number of field replicates (R1–3) that were classified as positive (+) or negative (-) for brown treesnake eDNA at four *Pandanus* plants, or sites (S1–4), located at four distinct locations (G1–4) and whether each of the 16 sites were considered present (1) or absent (0) for snake eDNA in subsequent occupancy modeling. Guam is considered fully occupied by brown treesnakes, and all sites had potential exposure to snake DNA.

	S1	S2	S3	S4
G1				
R1	+	-	-	-
R2	+	+	-	-
R3	-	-	+	-
Present	1	1	1	0
G2				
R1	-	-	+	-
R2	-	-	-	-
R3	-	+	+	+
Present	0	1	1	1
G3				
R1	-	-	-	-
R2	+	-	-	+
R3	-	-	-	-
Present	1	0	0	1
G4				
R1	+	-	-	-
R2	+	+	+	+
R3	-	+	+	-
Present	1	1	1	1

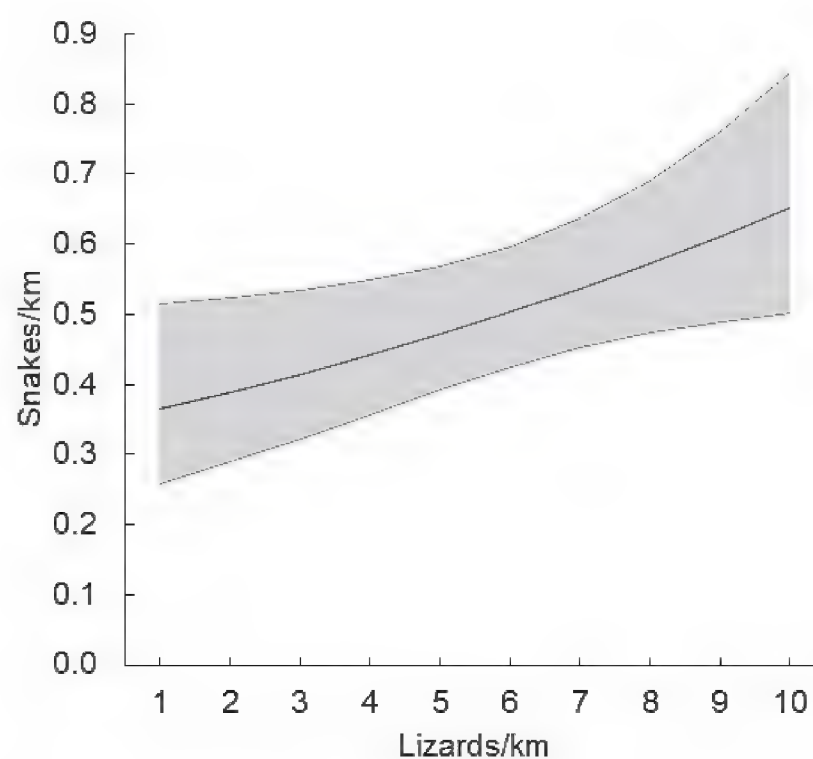


Figure 2. The predicted relationship between counts of lizards and brown treesnakes per km surveyed in Guam by trained observers from a Poisson generalized linear regression, with the 95% confidence interval in grey.

During the mock response, we completed 670 visual surveys and did not detect any brown treesnakes. Without accounting for variance in training level by individuals, sites had a > 99% probability that a snake would have been detected if there was a population density of at least three snakes per ha at each site ($d = 0.07 \pm 0.05$). Specific 95% CI site estimates for α are: Airport North [$k = 367$]: 1.00–1.00; Airport West [$k = 127$]: 0.994–1.00; and Seaport [$k = 166$]: 0.999–1.00.

Transect structure and pacing, Saipan

Elapsed times for completed surveys in 2016 ranged from 33–68 min, as compared to 20–36 min in 2023. The mean $\pm$ standardized deviation of pace in 2016 was 8.8 ± 2.7 m/min, compared to 9.3 ± 1.5 m/min in 2023. Within transects, the average deviation across all nights was 2.0 m/min in 2016 and 0.3 m/min in 2023, which was significantly smaller in 2023 than 2016 ($W = 102,400$, $P < 0.001$). Overall, thus, setting all transects to a single distance (300 m) that corresponded with an easy time-by-distance calculation (10 m/min) and visual cues easily marking distances drastically reduced variability in transect pace by observers across nights.

After controlling for the effects of observer and transect, a faster pace correlated significantly with an increased number of lizards counted by transect ($\beta = 0.05$, 95% CI = 0.02, 0.08, $z = 3.8$, $P < 0.001$). Continuously trained personnel counted twice the number of lizards on average compared to less or untrained observers ($\beta = -0.66$, 95% CI = -1.23 , -0.66 , $z = -2.2$, $P = 0.03$). There was, however, no evidence for an effect of an interaction between pace and observer training on the number of lizards detected ($\beta = -0.02$, 95% CI = -0.05 , 0.02 , $z = -0.8$, $P = 0.38$).

Effect of observer experience on cryptic reptile detection and multispecies bioinventories, Saipan

From all the 2023 Saipan transects, after controlling for observer, transect, and site effects, we found that years since Rapid Response Team training was inversely correlated with the number of lizards recorded ($\beta = -0.07$, 95% CI = -0.13 , -0.01 ,

$z = -2.3$, $P = 0.02$, Fig. 3A). The number of recent days on which an observer participated was a positive predictor of the number of lizards counted ($\beta = 0.10$, 95% CI = 0.09, 0.12, $z = 12.0$, $P < 0.001$), such that each additional day of participating in this exercise increased the number of lizards detected per transect by 1.1 times.

For non-cryptic prey items (birds and mammals), there was no relationship between years since training and counts ($\beta = 0.005$, 95% CI = -0.05 , 0.07 , $z = 0.2$, $P = 0.86$, Fig. 3B). Observers did, however, improve at detecting birds and mammals as the number of days they participated in observations increased ($\beta = 0.06$, 95% CI = 0.04 , 0.08 , $z = 3.2$, $P = 0.001$).

Searching for many species had a negative effect on the number of the target species recorded. Observers detected 1.2 times more emerald tree skinks on LS than ALL transects ($\beta = 0.22$, 95% CI = 0.14 , 0.31 , $z = 5.1$, $P < 0.001$), after controlling for site, transect, and observer effects. Years since prior training approached but was not significantly predictive of the number of LS recorded ($\beta = -0.05$, 95% CI = -0.11 , 0.01 , $z = -1.7$, $P = 0.09$). However, survey type and training were significantly interactive, such that the positive effect of searching for only one species was greater for people with increased years since Rapid Response Team training ($\beta = 0.03$, 95% CI = 0.11 , 0.15 , $z = 2.2$, $P = 0.02$). The number of days an observer participated in the mock response also had a positive effect on their detection of LS ($\beta = 0.13$, 95% CI = 0.005 , 0.07 , $z = 11.7$, $P < 0.001$). In other words, searching for a single, target species was most important for observers with no training, but all observers improved over time (Fig. 4).

Discussion

Overall, receiving specialized training to search for cryptic, arboreal reptiles was important for enhancing observer detection acuity. Given their high potential for ecological damage and substantial costs for control if they become established (Lowe et al. 2000), cryptic snakes represent strong candidate species for dedicated early detection and biosurveillance programs. However, their low detectability can cause challenges when developing protocols with reasonable error levels for assessing confidence in declaring absence. By applying a target analysis approach during a biosurveillance activity, we identified several protocol adjustments that may help

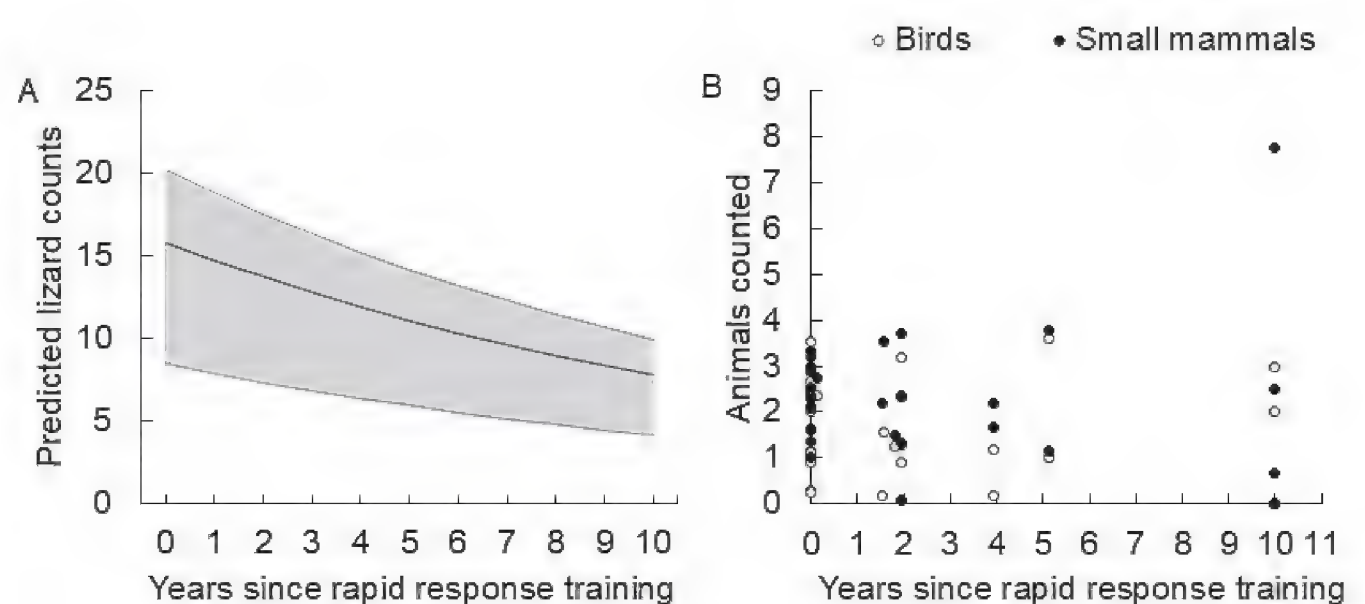


Figure 3. The predicted relationship between lizards (A) or the actual counts of small mammals and birds (B) counted by observers based on years since the observer underwent training for brown treesnake (*Boiga irregularis*) detection in Guam.

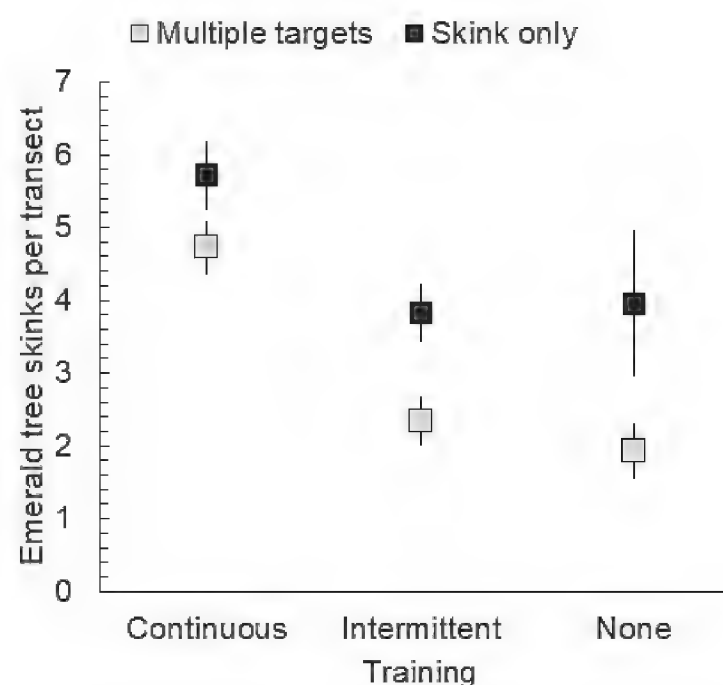


Figure 4. The average ($\pm$ standard error) number of emerald tree skinks counted by transect based on whether the surveyor was counting only the skinks or all lizards, small mammals, and birds (multiple targets) seen. Continuously trained observers completed roughly weekly surveys for brown treesnakes and lizards. Intermittently trained observers completed 25 h of training for brown treesnake surveys from 0.1–5.2 years ago. Untrained observers (none) had never received training.

improve standardization, detection probability, and accuracy of confidence levels during early detection of a cryptic invasive snake population.

Detection probability during visual surveys was density dependent for brown treesnakes, with lower density populations having lower detection probabilities. Densities are expected to be substantially lower in populations that are still early in the invasion curve, and thus low-density detection probability estimates are likely more accurate for use in forecasting confidence levels associated with no detection in snake-free islands. Differences in biosurveillance methods across locations, as well as density- or size-dependent detection probabilities, all introduce variability when estimating the probability of detecting an incipient population. In general, however, improved standardization of surveillance protocols that optimize survey approaches to maximize detection even at low densities should improve early detection and rapid response as a preventative tool for cryptic and costly invaders. Decision support approaches based on evaluation of target surveillance tools may facilitate development of frameworks for integrating less precise detection methods into decision-making and may enhance detection or reduce error associated with decisions.

The inclusion of eDNA into survey protocols may facilitate developing a robust early detection program for brown treesnakes, yet requires further testing and validation. Work on other cryptic reptiles has demonstrated that including eDNA in the sampling process, and particularly in conjunction with visual approaches, can improve detection probabilities overall (Matthias et al. 2021). The application of eDNA is, however, a relatively novel tool for terrestrial reptiles and complicated by false positives—the detection of a species that is not present—and false negatives—the failure to detect a species that is present (Wilcox et al. 2013; Evans et al. 2017; Nordstrom et al. 2022). Additionally, like visual surveys, detection probability when using eDNA tends to increase with organismal biomass in the environment (Piggott 2016). When using eDNA as a rapid response tool for invasive species, developing a clear decision support framework on when to act on a positive sample can improve application to decision-making. For instance, clear

establishment of risk tolerance from stakeholders for not responding when a positive detection is inaccurately equated to be a false positive versus the financial costs of responding further when species are actually absent can help formulate strategic response protocols (Sepulveda et al. 2022). Our data indicate that false negatives occurred in 65% of samples.

We found no evidence to support that brown treesnakes are established in Saipan. We generated relatively high confidence intervals that a snake would have been detected based on survey effort for detection probability estimates assuming an incipient population of 3–5 snakes per ha. Prior analyses of biosurveillance data have likewise suggested that there is not a population of brown treesnakes in Saipan, despite historical sightings on the island (Stanford and Rodda 2007; Yackel Adams et al. 2021). Regularity in conducting periodic biosurveillance exercises can help build evidence that informs the interpretation of uncertainty with respect to declaring Saipan free of brown treesnakes. However, these analyses do support that using lizards as a proxy may be a valid method to refine detection protocols when conducting biosurveillance activities for brown treesnakes on presumed snake-free islands. Thus, early detection initiatives can potentially be partnered with using approaches such as target analysis to test protocols aimed at reducing data noise and decreasing uncertainty associated with interpreting lack of detection as absence.

Standardization in surveys across observers, including untrained participants, was improved by setting consistent checkpoints based on intuitive timing along transects. Ensuring searchers could check their pace roughly every minute, expect to cover 10 m per min, and consistently require 30 min to complete a 300-m transect resulted in significantly less deviation in pace across observers and survey nights. Optimal search rates typically reflect tradeoffs between the rate at which an item or organism is encountered and the probability of detection when encountered (Gendron and Staddon 1983). Human observer pace has been documented to affect the probability of detecting a brown treesnake, with faster paces increasing the probability of detection at measured paces (Lardner et al. 2019b). Therefore, standardizing search speed is likely important when estimating or forecasting the presence or absence of the species. Variability in observer search protocols reduces the overall confidence in affiliated estimates by adding noise and increasing associated modeling error (Diefenbach et al. 2003). Although our results for detecting cryptic lizards were consistent with prior trends for brown treesnakes in Guam (Lardner et al. 2019b), one limitation of this dataset is that we compared data from two distinct years (2016 and 2023) to evaluate this effect. Thus, variability in environmental, climatic, or other causes that were not controlled for may also have influenced differences in the number of lizards counted. Regardless, improved standardization of protocols that requires less training to implement is likely to reduce error associated with statistical estimates of detection probability.

Focusing search efforts on fewer species also yielded a significant increase in the probability that an observer would detect the target species. This difference was particularly important for individuals with no training or with long periods since their last training had occurred. In studies of birds, detection ability improved with successive encounters of a single cryptic item, whereas searching for multiple items disrupted the development of a search image (Pietrewicz and Kamil 1979). Similar patterns have been identified for humans during visual surveys, where efficiency in locating items declines as the number of items searched for increases (Henke 1998). Our results supported that people improved their probability of detecting a target

organism by accumulating experience but also benefited from limiting the number of items or patterns they were required to identify. Optimal surveillance protocols may therefore include focused searching for only 1–2 similar species when species are difficult to detect, and especially when observers have little to no training.

We documented a large difference in ability to detect cryptic herpetofauna based on experience, with a continuously trained person detecting 50% more cryptic reptiles than a completely untrained observer on average. However, a limitation in fully interpreting our data is that most experienced observers had headlamps that exuded more than three times the light intensity, as measured by lumens, than most inexperienced observers. Prior research has suggested that a decrease in lumens of approximately four times resulted in 4.5 fewer snakes detected per transect, and similar conclusions have been found about lizards (Lardner et al. 2007; Lardner et al. 2015). The direct relationship between recent observation days and the number of lizards detected, which controlled for light differences, does, however, provide some independent support that training to create a search image enhanced detection regardless of headlamp light levels. Greater confidence in the difference between training and light levels would require replication where searchers of all training levels had equivalent lighting. Regardless, these data do suggest that maintaining a trained team of professional observers with adequate equipment may be an important consideration in establishing reliable biosurveillance programs for cryptic organisms.

Conclusion

We found that accuracy in estimates of the probability of brown treesnakes being absent could be improved by: (1) generating estimates of detection probability for the species in low-density environments; (2) accounting for training as a correction factor in generating detection probability estimates; and (3) creating transects that had easy-to-calculate and intuitive pacing structures. We also suggest that using lizards as a proxy for snakes when evaluating biosurveillance protocols in the Mariana Islands may have application in locations where snakes are not known to be present. Last, the terrestrial eDNA sampling protocol we developed for brown treesnakes shows promise for detecting snakes when present, albeit with a high false negative rate in the field. However, it remains to be determined if eDNA sampling can improve accuracy above visual surveys alone, has a density-dependent effect on detection probabilities, or is a cost-effective approach for this species.

Acknowledgements

We thank S. Igisomar and F. Masga for meeting with us and securing support from the Commonwealth of the Northern Mariana Islands Governor's Office to complete this work, as well as the many participants in this effort, with particular acknowledgment to the Commonwealth of the Northern Mariana Islands Division of Fish and Wildlife and Land and Natural Resources, the Center for Environmental Management of Military Lands and their funder, the Department of Navy, the U.S. Fish and Wildlife Service, the Research Corporation of the University of Guam, and the U.S. Geological Survey (USGS). We thank K. Courtot, C. Moore-Linn, and R. Reed for extensive logistical support with field implementation, as well as P. Barnhart for assistance with preparing eDNA sampling protocols. We

thank J. English, W. Daniels, and one anonymous reviewer for comments that improved earlier drafts of this manuscript. We also thank H. McCoy and the U.S. Office of Insular Affairs for financial administration and project support, as well as the USGS Biological Threats and Invasive Species Program for salary support. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

This work was supported by the Department of the Interior Office of Insular Affairs (Agreement #4500155266) and the USGS Biological Threats and Invasive Species Program.

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

MGN secured funding; MGN, MCV, SO, JF, WTD, and MP conceptualized the study; SO and JF designed the eDNA assay and completed lab work; MCV, MGN, JC, and WTD collected data; and MGN analyzed the data and prepared the first draft. All authors approved the manuscript.

Author ORCIDs

Melia G. Nafus  <https://orcid.org/0000-0002-7325-3055>

Marijoy C. Viernes  <https://orcid.org/0000-0002-9107-494X>

Data availability

Data are available for download:

– Yackel Adams and Lardner (2020): <https://doi.org/10.5066/P9QTSAHY>

– Nafus et al. (2023): <https://doi.org/10.5066/P9QRWKQB>

– Nafus and Viernes (2025): <https://doi.org/10.5066/P13JFSPU>

References

- Alvarez S, Solis D (2018) Rapid response lowers eradication costs of invasive species: Evidence from Florida. *Choices* 33: 1–9.
- Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effect models using Eigen and s4.
- Boback SM, Nafus MG, Yackel Adams AA, Reed RN (2020) Use of visual surveys and radiotelemetry reveals sources of detection bias for a cryptic snake at low densities. *Ecosphere* 11: e03000. <https://doi.org/10.1002/ecs2.3000>
- Caves EM, Jennings SB, HilleRisLambers J, Tewksbury JJ, Rogers HS (2013) Natural experiment demonstrates that bird loss leads to cessation of dispersal of native seeds from intact to degraded forests. *PLOS ONE* 8: e65618. <https://doi.org/10.1371/journal.pone.0065618>

- Christy MT, Yackel Adams AA, Rodda GH, Savidge JA, Tyrrell CL (2010) Modeling detection probabilities to evaluate management and control tools for an invasive species. *Journal of Applied Ecology* 47: 106–113. <https://doi.org/10.1111/j.1365-2664.2009.01753.x>
- Diefenbach DR, Brauning DW, Mattice JA (2003) Variability in grassland bird counts related to observer differences and species detection rates. *The Auk* 120: 1168–1179. [https://doi.org/10.1642/0004-8038\(2003\)120\[1168:VIGBCR\]2.0.CO;2](https://doi.org/10.1642/0004-8038(2003)120[1168:VIGBCR]2.0.CO;2)
- Doherty TS, Glen AS, Nimmo DG, Ritchie EG, Dickman CR (2016) Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences of the United States of America* 113: 11261–11265. <https://doi.org/10.1073/pnas.1602480113>
- Dorcas ME, Willson JD, Reed RN, Snow RW, Rochford MR, Miller MA, Meshaka WE, Andreadis PT, Mazzotti FJ, Romagosa CM, Hart KM (2012) Severe mammal declines coincide with proliferation of invasive Burmese Pythons in Everglades National Park. *Proceedings of the National Academy of Sciences of the United States of America* 109: 2418–2422. <https://doi.org/10.1073/pnas.1115226109>
- Epanchin-Niell RS, Hufford MB, Aslan CE, Sexton JP, Port JD, Waring TM (2010) Controlling invasive species in complex social landscapes. *Frontiers in Ecology and the Environment* 8: 210–216. <https://doi.org/10.1890/090029>
- Epanchin-Niell RS, Haight RG, Berec L, Kean JM, Liebhold AM (2012) Optimal surveillance and eradication of invasive species in heterogeneous landscapes. *Ecology Letters* 15: 803–812. <https://doi.org/10.1111/j.1461-0248.2012.01800.x>
- Evans N, Shirey P, Wieringa J, Mahon A, Lamberti G (2017) Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. *Fisheries (Bethesda, Md.)* 42: 90–99. <https://doi.org/10.1080/03632415.2017.1276329>
- Fosberg FR (1960) The vegetation of Micronesia. *Bulletin of the American Museum of Natural History* 119: 1–76.
- Fritts TH (2002) Economic costs of electrical system instability and power outages caused by snakes on the Island of Guam. *International Biodeterioration & Biodegradation* 49: 93–100. [https://doi.org/10.1016/S0964-8305\(01\)00108-1](https://doi.org/10.1016/S0964-8305(01)00108-1)
- Gendron RP, Staddon JER (1983) Searching for cryptic prey: The effect of search rate. *The American Naturalist* 121: 172–186. <https://doi.org/10.1086/284049>
- Goldberg CS, Pilliod DS, Arkle RS, Waits LP (2011) Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLOS ONE* 6: e22746. <https://doi.org/10.1371/journal.pone.0022746>
- Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP (2013) Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science* 32: 792–800. <https://doi.org/10.1899/13-046.1>
- Hauser CE, McCarthy MA (2009) Streamlining ‘search and destroy’: Cost-effective surveillance for invasive species management. *Ecology Letters* 12: 683–692. <https://doi.org/10.1111/j.1461-0248.2009.01323.x>
- Henke SE (1998) The effect of multiple search items and item abundance on the efficiency of human searchers. *Journal of Herpetology* 32: 112–115. <https://doi.org/10.2307/1565489>
- Hunter ME, Oyler-McCance SJ, Dorazio RM, Fike JA, Smith BJ, Hunter CT, Reed RN, Hart KM (2015) Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. *PLOS ONE* 10: e0121655. <https://doi.org/10.1371/journal.pone.0121655>
- Isaac NJB, van Strien AJ, August TA, de Zeeuw MP, Roy DB (2014) Statistics for citizen science: Extracting signals of change from noisy ecological data. *Methods in Ecology and Evolution* 5: 1052–1060. <https://doi.org/10.1111/2041-210X.12254>

- Jarić I, Heger T, Castro Monzon F, Jeschke JM, Kowarik I, McConkey KR, Pyšek P, Sagouis A, Essl F (2019) Crypticity in biological invasions. *Trends in Ecology & Evolution* 34: 291–302. <https://doi.org/10.1016/j.tree.2018.12.008>
- Kraus F (2015) Impacts from invasive reptiles and amphibians. *Annual Review of Ecology, Evolution, and Systematics* 46: 75–97. <https://doi.org/10.1146/annurev-ecolsys-112414-054450>
- Kyle KE, Allen MC, Dragon J, Bunnell JF, Reinert HK, Zappalorti R, Jaffe BD, Angle JC, Lockwood JL (2022) Combining surface and soil environmental DNA with artificial cover objects to improve terrestrial reptile survey detection. *Conservation Biology: The Journal of the Society for Conservation Biology* 36: e13939. <https://doi.org/10.1111/cobi.13939>
- Lardner B, Savidge JA, Rodda GH (2007) Spotting cryptic animals in the dark: What light properties should a good headlamp have? In: Witmer GW, Pitt WC, Fagerstone KA (Eds) *Managing Vertebrate Invasive Species: Proceedings of an International Symposium*. National Wildlife Research Center 2007, 234–245.
- Lardner B, Rodda GH, Yackel Adams AA, Savidge JA, Reed RN (2015) Detection rates of geckos in visual surveys: Turning confounding variables into useful knowledge. *Journal of Herpetology* 49: 522–532. <https://doi.org/10.1670/14-048>
- Lardner B, Yackel Adams AA, Knox AJ, Savidge JA, Reed RN (2019a) Do observer fatigue and taxon bias compromise visual encounter surveys for small vertebrates? *Wildlife Research* 46: 127–135. <https://doi.org/10.1071/WR18016>
- Lardner B, Yackel Adams AA, Savidge JA, Reed RN (2019b) Optimizing walking pace to maximize snake detection rate: A visual encounter survey experiment. *Herpetologica* 75: 218–223. <https://doi.org/10.1655/D-18-00020>
- Larson ER, Graham BM, Achury R, Coon JJ, Daniels MK, Gambrell DK, Jonassen KL, King GD, LaRacunte N, Perrin-Stowe TI, Reed EM, Rice CJ, Ruzi SA, Thairu MW, Wilson JC, Suarez AV (2020) From eDNA to citizen science: Emerging tools for the early detection of invasive species. *Frontiers in Ecology and the Environment* 18: 194–202. <https://doi.org/10.1002/fee.2162>
- Lebreton J-D, Gimenez O (2013) Detecting and estimating density dependence in wildlife populations. *The Journal of Wildlife Management* 77: 12–23. <https://doi.org/10.1002/jwmg.425>
- Lindén A, Mäntyniemi S (2011) Using the negative binomial distribution to model overdispersion in ecological count data. *Ecology* 92: 1414–1421. <https://doi.org/10.1890/10-1831.1>
- Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B, Reichard S, Mack RN, Moyle PB, Smith M, Andow DA, Carlton JT, McMichael A (2006) Biological invasions: Recommendations for U.S. policy and management. *Ecological Applications: A Publication of the Ecological Society of America* 16: 2035–2054. [https://doi.org/10.1890/1051-0761\(2006\)016\[2035:BIRFUP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2)
- Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species: A selection from the global invasive species database. The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). <https://portals.iucn.org/library/sites/library/files/documents/2000-126.pdf>
- MacKenzie DI, Nichols JD, Hines JE, Knutson MG, Franklin AB (2003) Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology* 84: 2200–2207. <https://doi.org/10.1890/02-3090>
- Marbuaah G, Gren I-M, McKie B (2014) Economics of harmful invasive species: A review. *Diversity* 6: 500–523. <https://doi.org/10.3390/d6030500>
- Matthias L, Allison MJ, Maslovat CY, Hobbs J, Helbing CC (2021) Improving ecological surveys for the detection of cryptic, fossorial snakes using eDNA on and under artificial cover objects. *Ecological Indicators* 131: 108187. <https://doi.org/10.1016/j.ecolind.2021.108187>
- McArdle B (1990) When are rare species not there? *Oikos* 57: 276–277. <https://doi.org/10.2307/3565950>

- Mehta SV, Haight RG, Homans FR, Polasky S, Venette RC (2007) Optimal detection and control strategies for invasive species management. *Ecological Economics* 61: 237–245. <https://doi.org/10.1016/j.ecolecon.2006.10.024>
- Mollot G, Pantel JH, Romanuk TN (2017) The effects of invasive species on the decline in species richness: A global meta-analysis. In: Bohan DA, Dumbrell AJ, Massol F (Eds) *Advances in Ecological Research*. Academic Press, 61–83. <https://doi.org/10.1016/bs.aecr.2016.10.002>
- Morisette JT, Reaser JK, Cook GL, Irvine KM, Roy HE (2020) Right place. Right time. Right tool: Guidance for using target analysis to increase the likelihood of invasive species detection. *Biological Invasions* 22: 67–74. <https://doi.org/10.1007/s10530-019-02145-z>
- Nafus MG, Viernes MC (2025) Saipan, data collected from visual surveys during a mock rapid response exercise, October 2023. U.S. Geological Survey data release. <https://doi.org/10.5066/P13JFSPU>
- Nafus MG, Savidge JA, Yackel Adams AA, Christy MT, Reed RN (2018) Passive restoration following ungulate removal in a highly disturbed tropical wet forest devoid of native seed dispersers. *Restoration Ecology* 26: 331–337. <https://doi.org/10.1111/rec.12559>
- Nafus MG, Mazzotti FJ, Reed RN (2020) Estimating detection probability for Burmese pythons with few detections and zero recaptures. *Journal of Herpetology* 54: 24–30. <https://doi.org/10.1670/18-154>
- Nafus MG, Collins AE, Viernes MC, Hopkins CB, Nacpil A (2023) Guam, USGS Closed Population (NWFN), an experimental eradication of brown treesnakes in a 5-ha study site, 2016–2023. U.S. Geological Survey data release. <https://doi.org/10.5066/P9QRWKQB>
- Nafus MG, Reyes A, Fies T, Goetz SM (2024) Adaptive resource management (ARM): Achieving functional eradication of invasive snakes to benefit avian conservation. *Journal of Applied Ecology* 61: 733–745. <https://doi.org/10.1111/1365-2664.14597>
- Nordstrom B, Mitchell N, Byrne M, Jarman S (2022) A review of applications of environmental DNA for reptile conservation and management. *Ecology and Evolution* 12: e8995. <https://doi.org/10.1002/ece3.8995>
- Pietrewicz AT, Kamil AC (1979) Search image formation in the blue jay (*Cyanocitta cristata*). *Science* 204: 1332–1333. <https://doi.org/10.1126/science.204.4399.1332>
- Piggott MP (2016) Evaluating the effects of laboratory protocols on eDNA detection probability for an endangered freshwater fish. *Ecology and Evolution* 6: 2739–2750. <https://doi.org/10.1002/ece3.2083>
- Piquet JC, López-Darias M (2021) Invasive snake causes massive reduction of all endemic herpetofauna on Gran Canaria. *Proceedings of the Royal Society B, Biological Sciences* 288: 20211939. <https://doi.org/10.1098/rspb.2021.1939>
- Pitt W, Vice DS, Pitzler M (2005) Challenges of invasive reptiles and amphibians. In: Nolte DL, Fagerstone KA (Eds) *Proceedings of the 11th Wildlife Damage Management Conference*. The Wildlife Society, 2005, 112–119.
- Roberts M, Cresswell W, Hanley N (2018) Prioritising invasive species control actions: Evaluating effectiveness, costs, willingness to pay and social acceptance. *Ecological Economics* 152: 1–8. <https://doi.org/10.1016/j.ecolecon.2018.05.027>
- Rodda GH, Savidge JA (2007) Biology and impacts of Pacific Island invasive species. 2. *Boiga irregularis*, the brown tree snake (Reptilia: Colubridae). *Pacific Science* 61: 307–324. [https://doi.org/10.2984/1534-6188\(2007\)61\[307:BAIOPI\]2.0.CO;2](https://doi.org/10.2984/1534-6188(2007)61[307:BAIOPI]2.0.CO;2)
- Rodda GH, Savidge JA, Tyrrell CL, Christy MT, Ellingson AR (2007) Size bias in visual searches and trapping of brown treesnakes on Guam. *The Journal of Wildlife Management* 71: 656–661. <https://doi.org/10.2193/2005-742>
- Rose JP, Wademan C, Weir S, Wood JS, Todd BD (2019) Traditional trapping methods outperform eDNA sampling for introduced semi-aquatic snakes. *PLOS ONE* 14: e0219244. <https://doi.org/10.1371/journal.pone.0219244>

- Savidge JA (1987) Extinction of an island forest avifauna by an introduced snake. *Ecology* 68: 660–668. <https://doi.org/10.2307/1938471>
- Sepulveda A, Smith D, O'Donnell K, Owens N, White B, Richter C, Merkes C, Wolf S, Rau M, Neilson M, Daniel W, Dumoulin C, Hunter M (2022) Using structured decision making to evaluate potential management responses to detection of dreissenid mussel (*Dreissena* spp.) environmental DNA. *Management of Biological Invasions: International Journal of Applied Research on Biological Invasions* 13: 344–368. <https://doi.org/10.3391/mbi.2022.13.2.06>
- Silva-Rocha I, Salvi D, Sillero N, Mateo JA, Carretero MA (2015) Snakes on the Balearic Islands: An invasion tale with implications for native biodiversity conservation. *PLOS ONE* 10: e0121026. <https://doi.org/10.1371/journal.pone.0121026>
- Stanford JW, Rodda GH (2007) The brown treesnake Rapid Response Team. Witmer GW, Pitt WC, Fagerstone KA (Eds) *Managing Vertebrate Invasive Species: Proceedings of an International Symposium*. National Wildlife Research Center 2007, 175–217.
- Tobin ME, Sugihara RT, Pochop PA, Linnell MA (1999) Nightly and seasonal movements of *Boiga irregularis* on Guam. *Journal of Herpetology* 33: 281–291. <https://doi.org/10.2307/1565725>
- Vice DS, Pitzler M (2000) Brown treesnake control: Economy of scales. In: Clark L, Hone J, Shivik JA, Watkins RA, VerCauteren KC, Yoder JK (Eds) *Proceedings of the Third NWRC Special Symposium. Human Conflicts with Wildlife: Economic Considerations*. National Wildlife Research Center, 2000, 127–131.
- Vine SJ, Crowther MS, Lapidge SJ, Dickman CR, Mooney N, Piggott MP, English AW (2009) Comparison of methods to detect rare and cryptic species: A case study using the red fox (*Vulpes vulpes*). *Wildlife Research* 36: 436–446. <https://doi.org/10.1071/WR08069>
- Wilcox TM, McKelvey KS, Young MK, Jane SF, Lowe WH, Whiteley AR, Schwartz MK (2013) Robust detection of rare species using environmental DNA: The importance of primer specificity. *PLOS ONE* 8: e59520. <https://doi.org/10.1371/journal.pone.0059520>
- Willson JD (2017) Indirect effects of invasive Burmese pythons on ecosystems in southern Florida. *Journal of Applied Ecology* 54: 1251–1258. <https://doi.org/10.1111/1365-2664.12844>
- Yackel Adams AA, Lardner B (2020) Visual survey rapid response Saipan 2016. U.S. Geological Survey data release. <https://doi.org/10.5066/P9QTSAHY>
- Yackel Adams AA, Lardner B, Knox AJ, Reed RN (2018) Inferring the absence of an incipient population during a rapid response for an invasive species. *PLOS ONE* 13: e0204302. <https://doi.org/10.1371/journal.pone.0204302>
- Yackel Adams AA, Barnhart PD, Rodda GH, Hileman ET, Nafus MG, Reed RN (2021) Can we prove that an undetected species is absent? Evaluating whether brown treesnakes are established on the island of Saipan using surveillance and expert opinion. *Management of Biological Invasions* 12: 901–926. <https://doi.org/10.3391/mbi.2021.12.4.09>
- Yeo M-H, Patil UD, Chang A, King R (2023) Changing Trends in Temperatures and Rainfalls in the Western Pacific: Guam. *Climate (Basel)* 11: 81. <https://doi.org/10.3390/cli11040081>